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INTRODUCTION

The project is intended to determine whether a specific nitric oxide-mediated tumor stress response pathway that is initiated following a cytotoxic injury (1) is observed in prostate cancer xenografts and (2) can be inhibited by the administration of dietary glycine supplementation. The rationale is based upon prior preclinical studies establishing, for other solid tumor types, that upregulation of inducible nitric oxide synthase (iNOS) in activated macrophages recruited to the site of cytotoxic injury from radiation or chemotherapy leads to the production of NO that stabilizes hypoxia-inducible factor 1- α , which in turns leads to increased expression of vascular endothelial growth factor (VEGF). As a result of this signaling process, tumor angiogenesis is supported, leading to recovery from the initial cytotoxic injury. It is believed that inhibiting this NO-mediated process of angiogenesis can enhance the cytotoxicity of radiation or chemotherapy, and it is believed that glycine might be an effective

BODY

The tasks outlined in the approved statement of work are as listed below, followed by the research accomplishments to date for each task

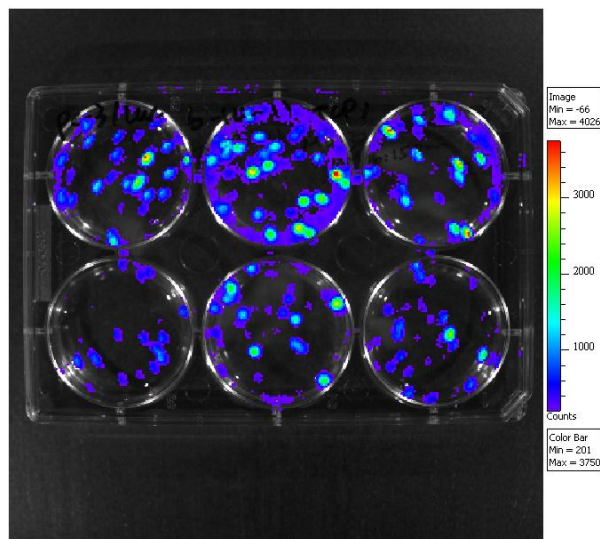
Task 1. PREPARATION FOR XENOGRAFT STUDIES

1a. Preparation, submission, and review of animal protocol by institutional committee for review of animal studies

This task was successfully accomplished, and the University of Colorado Animal Care and use Committee continues to monitor the animal protocol activities. A copy of the approved animal protocol has already been submitted but is available upon additional request if desired. The institutional reference number is 83511(02)1E, and the title is the same as the overall project title.

1b. Transfection of prostate cancer cell lines (PC3, LNCaP) with Hif-1 α reporter gene (firefly luciferase-based); validation in vitro with bioluminescence methods

This task has also been accomplished. The figure to the right is a representative image of the in vitro growth and bioluminescence imaging of PC3 cells transfected with the Hif-1 α reporter gene. The colonies with activity indicated on the associated color spectrum as trending toward the red side of the spectrum corresponded to cells that were successfully transfected. These colonies with high reporter uptake were selectively harvested and then expanded and maintained as stock in frozen form, from which aliquots were thawed and re-expanded as needed for injection into the murine hosts for radiation experiments.



LnCAP cells were similarly successfully transfected (image not shown). However, as noted below, the LnCAP cell line proved less well suited for the xenograft studies as a result of the much slower in vitro and in vivo growth kinetics.

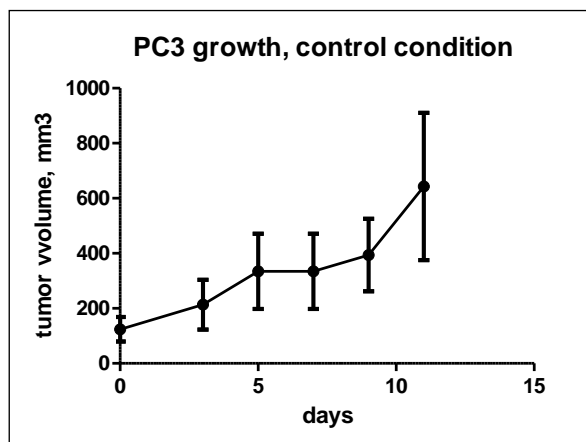
Task 2. XENOGRAFT STUDIES FOR SPECIFIC AIM 1: To determine whether radiation-induced cytotoxicity triggers a response in prostate cancer xenografts involving macrophage-mediated stabilization of Hif-1 α and upregulation of VEGF

2a. Establish control growth rates for transfected cell lines

Implanted transfected LnCAP cells required >4 weeks to grow to a palpable state, and this time frame did not appear feasible relative to the overall project. Thus, efforts were concentrated upon PC3 cells.

A representative growth curve after implantation of cells in matrigel into the flank of the host animals (mice) is shown in the figure to the right, with measurements taken beginning with the first day tumors were palpable, in this experiment 7 days implantation.

Note that in this experiment and in all others, the veterinarian in the small animal facility evaluates animals frequently for their overall status and sacrifice when the tumor growth has caused substantial distress to the animal. The maximum volume reached before this assessment is generally in the range of 2000 mm³ or larger.



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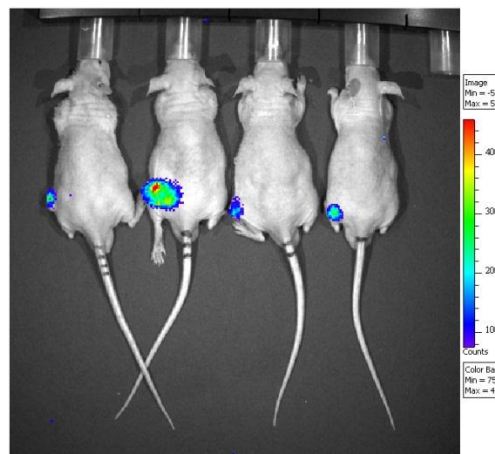
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2b. For selected inoculum, irradiation (0, 2, 6 Gy) when palpable tumor and bioluminescence imaging of Hif-1 α

The PC3 cells transfected with the Hif-1 α reporter gene were well imaged using the bioluminescence system. A representative example of one of the imaged animal groups shown in the figure to the right.

The images may be analyzed to quantify the determine the amount of Hif-1 α expression, as determined by the total of counts detected after 1 minute exposure via bioluminescence assay. A representative example of one data is shown below:

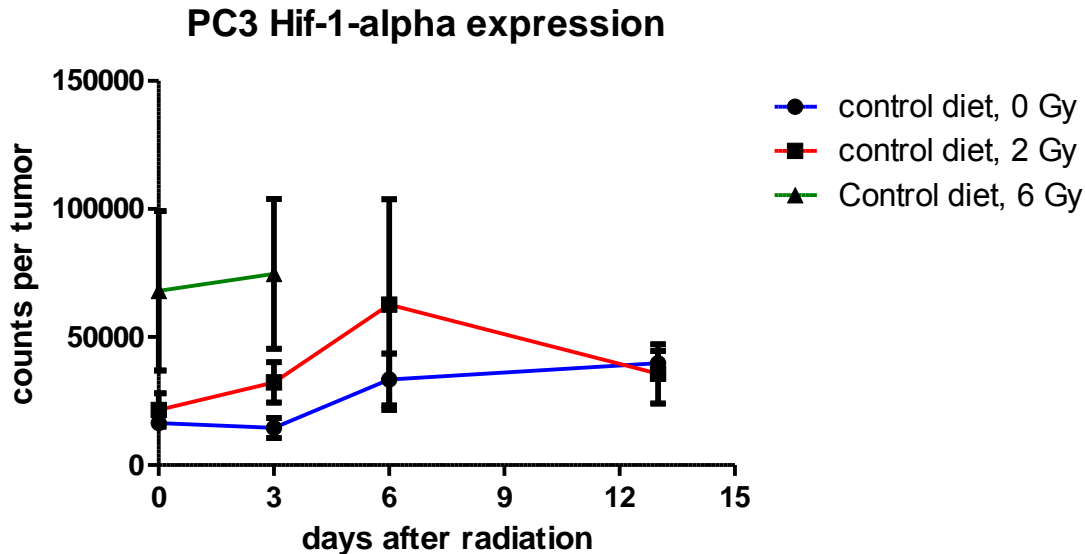


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In the example shown, radiation-induced Hif-1 α expression peaked 6 days after a 2 Gy dose and then declined. The 6 Gy group was invaluable because the tumors grew rapidly, and the animals had to be sacrificed. In this early experiment, the 6 Gy group was selected by choosing animals with higher tumor volume at time 0 prior to radiation exposure, in an effort to enhance the observation of Hif-1 α expression, since the main purpose of this initial experiment was to observe a possible dose-response relationship between radiation dose and magnitude of Hif-1 α expression. In future experiments, it was appreciated that it was better to irradiate tumors at a smaller initial volume, and dose groups were balanced with respect to average baseline tumor volume.

2c. For optimal dose showing effect in 2b, study of macrophage depletion using carrageenan treatment: control vs irradiated vs carrageenan vs irradiated+carrageenan

A single carrageenan experiment was performed, whereby 4 animals received 0, 3, or 6 Gy radiation dose after tumor implantation and growth to palpable status. The results clearly indicated that carrageenan effectively eliminated the increased Hif-1 α expression observed after radiation in control conditions (data not shown). However, it was also readily apparent that the carrageenan also appeared to put the animals at risk for a generally compromised overall health condition, presumably as a result of susceptibility to infection as a result of immune system compromise.

Task 3. XENOGRAFT STUDIES FOR SPECIFIC AIM 2: To determine whether inhibition of macrophage iNOS using a known iNOS inhibitor (L-NAME) or dietary glycine blocks radiation-mediated Hif-1 α and enhances radiation-mediated tumor growth delay in prostate cancer xenografts.

3a. Bioluminescence imaging of Hif-1 α : control v L-NAME v dietary glycine, all irradiated

3b. Tumor growth delay: control vs glycine vs irradiation vs irradiation+glycine

This experiment is currently ongoing in the no-cost extension period and will be completed and analyzed within the next two months.

KEY RESEARCH ACCOMPLISHMENTS:

- successful transfection of PC3 cells with Hif-1 α bioluminescent reporter
- implantation of transfected cells into nude mice and characterization of growth rates under control conditions
- characterization of radiation-induced increase in Hif-1 α expression in vivo in PC3 tumors
- abrogation of this increased Hif-1 α expression with the use of carrageenan, establishing that the pathway likely involves macrophages

REPORTABLE OUTCOMES:

- We anticipate submission of an abstract to next year's ASTRO meeting and a submission of a manuscript to the International J of Radiation Oncology, Biology, Physics or equivalent journal with a readership interested in this topic.

CONCLUSION:

The experiments performed to date support the feasibility of studying Hif-1 α expression in vivo in prostate cancer xenografts. We have established that there is an observable increase in Hif-1 α expression after ionizing radiation, and we are now addressing the pivotal question of the project with experiments currently underway. Specifically, if we observe that supplemental dietary glycine (1) blocks Hif-1 α expression and (2) thereby produces growth delay after radiation, then this finding will support application for additional funding to translate the observation into an early phase clinical trial.